

FOLLOW-UP MEASURE

PRELIMINARY ASSESSMENT REPORT

MUTUAL RECOGNITION

Invented name of the pharmaceutical product in the Reference Member State	Prozac
Name(s) of the active substances and strength	Fluoxetine
Pharmacotherapeutic classification	ATC code NO6A BO3 Selective Serotonin Reuptake Inhibitor (SSRI)
Pharmaceutical form	Capsules, 20 mg fluoxetine Oral solution, 20 mg fluoxetine per 5 ml

Reference No. for the Mutual Recognition Procedure	UK/H/636/01,03/II/02
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In the Reference Member State: UK

Marketing authorisation holder's name and address.	Eli Lilly and Company limited Kingsclere Road, Basingstoke Hampshire, RG21 6XA UNITED KINGDOM
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Name, telephone and telefax number of contact point in the Member State for discussion on issues raised by the Assessment Report (where applicable)	
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Date of FAR (Day 40)	
Deadline for comment by CMS (Day 55)	

Nature of change requested	Follow up measure: To assess the findings of juvenile toxicity studies conducted to investigate the effect of Fluoxetine (Prozac) on emotional behaviour
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PRELIMINARY EXECUTIVE SUMMARY

Product Name: Prozac (UK/H/636/01, 03/II/02)

Follow up measure: To assess the findings of juvenile toxicity studies conducted to investigate the effect of Fluoxetine (Prozac) on emotional behaviour in rodents.

I. INTRODUCTION

On 1 June 2006, a positive opinion was adopted by the CHMP for the use of fluoxetine in the EU for the treatment of major depressive episodes unresponsive to psychological therapy after 4–6 sessions in children aged 8 to 17 years.

Some pre-clinical issues remained unresolved at the time of the opinion. A study published by de Jong and colleagues (2006) reported that chronic treatment of rats with the SSRIs fluvoxamine and paroxetine during adolescence resulted in changes in tests designed to measure emotional and sensory gating. The purpose of this study was to evaluate whether administration of fluoxetine HCL to rats during the same period affected behavioural performance on the same tests: anxiety-like behaviour in the elevated plus maze, depressive-like behaviour in the forced swimming tests, and sensory gating in the pre-pulse inhibition of acoustic startle test. As paroxetine has been shown to produce effects on these types of behaviours under similar conditions, it was included in this study as a comparator.

As part of the CHMP conditions of approval of the paediatric indication, the Marketing Authorisation Holder (MAH) agreed a risk management plan which included a commitment to investigate the effect of fluoxetine hydrochloride on emotional behaviour of juvenile CD rats and to determine their relevance to the intended paediatric patient population. They also committed to co-operate with clinical investigators in developing a NIMH sponsored study in children. The MAH submitted draft protocols for the following studies for assessment on 30th June 2006 and the concerned member states agreed the final protocol for the study on October 2006:

- **Characterise the effect of fluoxetine hydrochloride on specified emotional behaviours in the juvenile rat.**

The study on emotional behaviours has now been completed and the MAH has submitted the final study report for assessment.

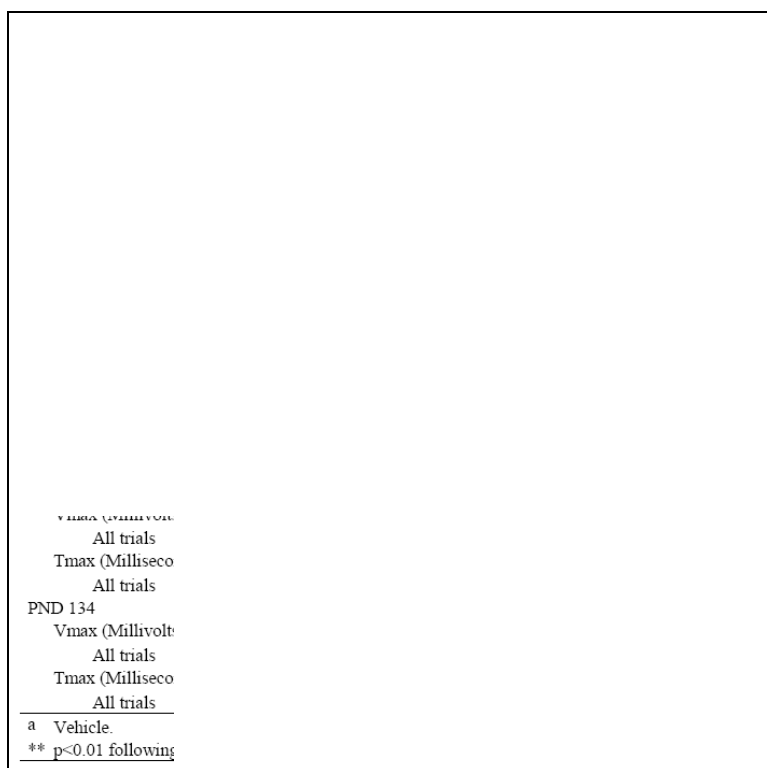
II. BACKGROUND TO THIS PROCEDURE: THE SSRIs AND EMOTIONAL BEHAVIOUR

Concerns regarding the potential effects of fluoxetine and the SSRIs on emotional behaviour in the paediatric population was raised during the assessment of the variation application for the paediatric indication of Fluoxetine in Major Depressive Episodes (UK/H/636/1,3/II/002) and the subsequent ***Referral under Article 6(12) of Commission Regulation (EC) N 1084/2003 (EMEA/H/A-6(12)/671)*** in response to findings in rodent studies showing treatment related increase in anxiogenic effect in juvenile animals which is in contrast to its intended pharmacological action – anxiolysis as observed in adult non-clinical and clinical studies. In addition impaired performance in memory and learning tests was observed in juvenile rodents as outlined below:

2.1 In a behavioural toxicity study by **de Jong et al (2006)**, potential effects of SSRIs on the developing serotonergic system was investigated in adult male Wistar rats that had been treated throughout the **period of adolescence** and then evaluated in tests of emotional and sensory gating. Male rats treated with 30mg/kg/day **fluvoxamine** and 15mg/kg/day **paroxetine** showed a reduction in ejaculation frequency and in time spent in the open arm of the elevated plus maze. Fluvoxamine also slightly increased avoidance latency in the elevated T-maze compared to paroxetine. Overall the SSRIs led to an increase in anxiety like behaviours.

The study also investigated the functioning of the 5-HT 1A receptor agonist 8-OH-DPAT (0.0, 0.1, 0.2, and 0.4 mg/kg sc) as previous tests have showed an association between modulation of the serotonergic system and behaviour of rats in the elevated T- maze and the elevated plus maze leading some to believe that sustained exposures to SSRIs during adolescence could lead to a sustained change in 5-HT neurotransmission or functioning of the 5-HT receptors involved in anxiety like behaviours. The study showed that injection of the 5-HT 1A receptor agonist 8- OH-DPAT had no long-term effect on functioning as there was no effect on lower lip retraction or ejaculation frequency **de Jong et al (2006)**.

2.2 In another behavioural toxicity study by [REDACTED] *3, 10 or 30 mg/kg/day Fluoxetine hydrochloride was administered to **juvenile** CD rats for 70 days (10 weeks $\pm$,) by oral gavage. The performance of the rats in the following behavioural test paradigms was evaluated 2 and 6 weeks after the cessation of treatment: acoustic startle test (anxiety test), Biel Maze (learning) and locomotor activity (motor effects).



* the daily dose for children is 10 - 40 mg, which is equivalent to 0.4 – 1.6 mg/kg/day for a 25 kg child

$\pm$ The period of treatment selected for this study was intended to be “equivalent” to treatment of humans from 2 to 16/18 years of age.

The results of the study showed a dose-dependent reduction in startle response (reduced amplitude, and increased latency) relative to control in male and female rats at all doses tested [statistically significant at 30/mg/kg/day] during behavioural tests performed 2 weeks post-treatment (**PND 106**). **However, these** returned back to control values 4 weeks later (PND 134) in the low and intermediate groups but not in the high dose (30 mg/kg/day) group. Behavioural effects were also observed in the Biel Maze test. In addition the time to complete the **water maze**, and the number of errors were significantly increased in the learning phase of the trial for the 30 mg/kg/day females 2 weeks after cessation of treatment.

Overall the findings of this study indicated that fluoxetine was potentially anxiogenic in juvenile rats and had an effect on learning. The authors speculated that the effect in the low and intermediate dose groups was a pharmacological response as opposed to a permanent chemical modification. The effects at the top dose had not demonstrated reversibility nevertheless this dose is associated with significant toxicity and is not relevant to clinical exposures of fluoxetine

2.3 Long-term behavioural effects were also observed in a study conducted by **Ansorge et al 2004** in **mice** treated between postnatal day 4-21 with fluoxetine hydrochloride. In this study, the phenotypic behaviour of wild type mice with respect to the serotonin transporter gene (5-HTT^{+/+}), was compared with the behaviours of mutant 5HTT knock out mice (5-HTT^{-/-}) and heterozygous mice (5-HTT^{+/-}) before and after treatment with fluoxetine (10mg/kg/day i.p.) from postnatal day 4-21. The behavioural tests which evaluated exploratory behaviour, anxiety-related and depression related behaviours and shock avoidance behaviours, were performed 9 weeks post-treatment and showed treatment-related change in the behaviours of the heterozygous and wild type groups, towards that characteristic of the knock-out phenotype. Fluoxetine had no effect on the knockout mice. The authors concluded that exposure to fluoxetine during CNS development led to long-term/permanent effects on adult behaviour which could have implications for the efficacy of treatment administered during adulthood. Although the reversibility of these effects was not specifically investigated the fact that they were present 9 weeks post-treatment indicated a prolonged effect.

The age range of the treated animals is equivalent to the third trimester of pregnancy through to 2 years of age in humans which is not relevant to the age range of the indicated paediatric population therefore these findings are not considered to be clinically relevant. Although it does provide some insight into the relationship between chronic fluoxetine treatment and the expression of the receptors involved in serotonergic neurotransmission.

II.1 CHMP conclusion on the SSRIs and emotional behaviour

The CHMP reviewed these study findings and concluded that the anxiogenic effects observed in the studies by Ansorge et al. 2004 and de Jong et al. 2006 were minor, specific to individual assessments and/or not relevant to the patient population because the studies were either conducted in genetically manipulated transgenic animal models of depressions/ anxiety with dosing periods not relevant to the age-range of the indicated paediatric population. In addition, the most serious finding - the non-reversible changes in startle and learning in the female rats in the [REDACTED] study only occurred in conjunction with frank/overt toxicity thus making it impossible to distinguish between direct effect of toxicity or a secondary effect of the toxicity elicited by the high dose. In addition, the exposures associated with the latter finding are not clinically relevant. The MAH argued about the difficulty of extrapolating findings from the animal behavioural studies to the human situation because of a number of factors namely the range of contrasting responses to fluoxetine in the pre-clinical data, the apparent

effect of strain and different behavioural tests on the outcome of the study. The fact that the tests are performed either in healthy rats or chemically/genetically induced models of anxiety/depression which is not equivalent to the brain function of a depressed child/adolescent and lastly the difficulty in investigating adverse effects of behaviour in a depressed adolescent population – i.e. lack of adequate controls, the influence of external factors –confounders. Nevertheless the CHMP felt the findings of the pre-clinical studies warranted further investigation in a non-clinical study conducted in the appropriate aged rats using concentrations that do not elicit frank toxicity to distinguish between secondary effect of toxicity and primary effects on the CNS.

They recommended that a further pre-clinical study should be performed and the design of the study should be such that the age/development phase of the animals when exposed is as closely matched to the development of the brain in the target paediatric population. The focus of the assessment was to be on measures of anxiety.

The protocol for this study was assessed in a mutual recognition follow up measure and the final protocol was agreed between the RMS, CMS and the MAH in 2006. The MAH has now submitted the final report of the study for assessment which is discussed below.

III. BEHAVIOURAL STUDY IN JUVENILE RATS

1. [REDACTED] 2008. An Emotional Behaviour Study in Sprague Dawley CD rats given fluoxetine or Paroxetine daily by gavage from Postnatal Day (PND) 33 To 62

Study outline

The purpose of this study was to investigate the effect of fluoxetine on the emotional behaviour of male juvenile Sprague Dawley rats. The study is mechanistic and thus not conducted to GLP. Fluoxetine HCl or paroxetine HCl was administered by oral gavage to two separate groups of juvenile rats from PND 33 to 62. Group A was tested for behavioural performance during the dosing period (initiated on PND 57), while Group B was tested approximately 2 months after dosing ceased (initiated on PND 122). Each group consisted of 20 male rats selected such that only 1 male per treatment was taken from any one litter (within litter design). Control rats received purified water. **Fluoxetine HCl** doses were **3 and 10 mg/kg** (expressed as the freebase and adjusted for purity) as transient effects on acoustic startle response was previously observed at these doses([REDACTED] 2004). **Paroxetine HCl** doses were **3, 10, and 17 mg/kg** (expressed as the freebase and adjusted for purity). The 17 mg/kg dose of paroxetine HCl was selected to match the 15 mg/kg paroxetine (base) dose given in the [de Jong study](#).

Live phase parameters included, clinical signs (daily for mortality/morbidity); body weights (weekly except during dosing treatment period which was daily) and measurement of plasma concentrations of corticosterone at terminal sacrifice. The behavioural test battery included the elevated plus-maze (on PND 57 and PND 122), prepulse inhibition of startle response (PND 59 and PND 124), forced swimming test (group A=PND 61 and 62; group b=PND 126 and 127) as agreed by the RMS and CMS during the finalising of the study protocol.

Results

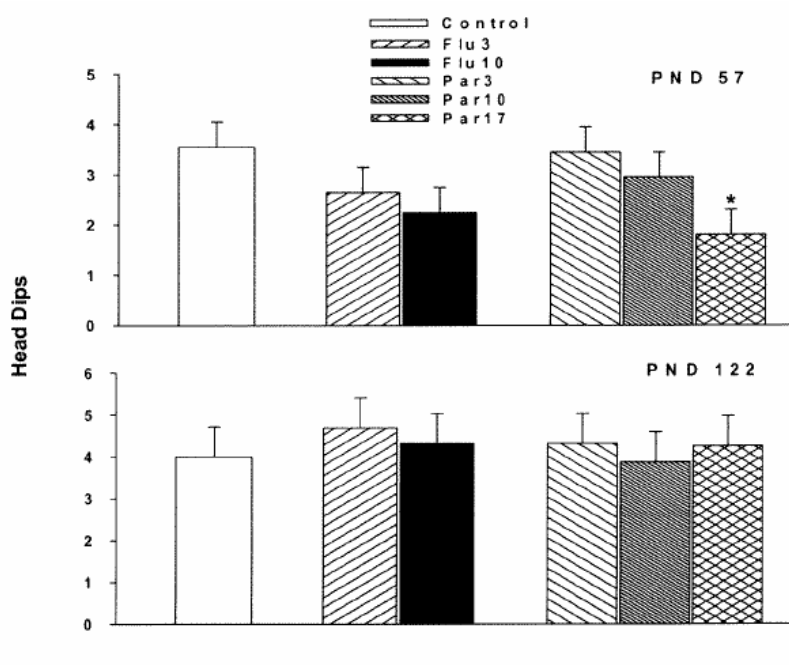
Body Weights

Body weights were only minimally decreased in the 10-mg/kg fluoxetine rats and the 17-mg/kg paroxetine rats (Figures 1-3). Decreases in body weights generally occurred during the second week of dosing through to the end of the dosing period. However, by the second week of the post-treatment

period, body weights of all treatment groups were comparable to controls, demonstrating complete reversibility (Figure 4).

Elevated plus-maze – assay of anxiety-related responses of rodents

Treatment with fluoxetine did not affect performance in the elevated plus-maze. All parameters evaluated were similar to control values. The 17-mg/kg paroxetine rats demonstrated significantly reduced numbers of head dips (Figure 6) and, although not statistically significant, less time in the open arms of the maze when tested during the dosing period (Figure 5). This anxiogenic behaviour is consistent with the findings reported by de Jong and colleagues (2006) and thus reproducible. Latency to enter the open arms was unaffected during the dosing period (Figure 7). There were no effects in the paroxetine treated rats when tested during the post-treatment period.



Prepulse inhibition of startle response

Treatment with fluoxetine or paroxetine did not affect prepulse inhibition of the startle response (Figures 8 and 9).

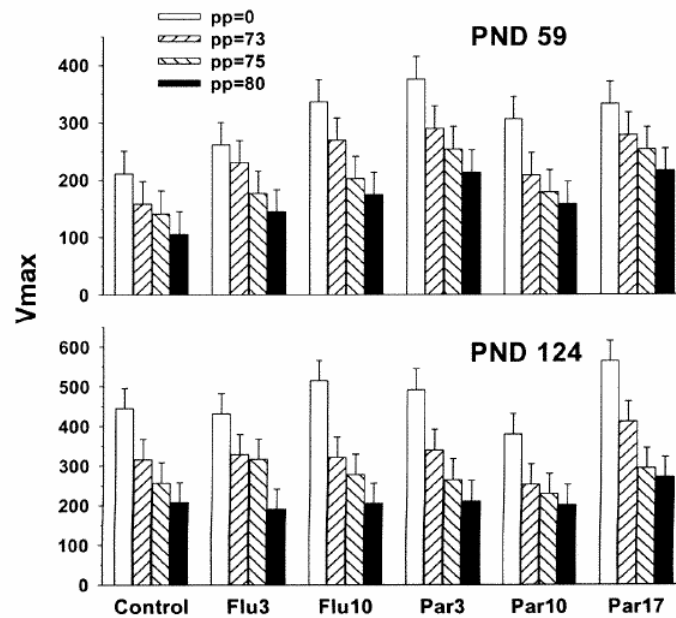


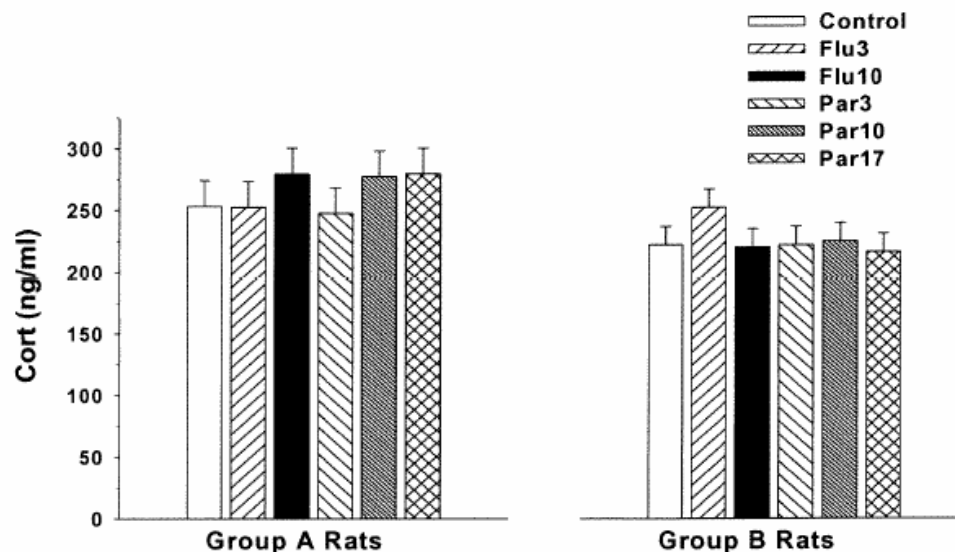
Figure 8: Acoustic startle/Prepulse inhibition test. Vmax is the maximum startle amplitude measured during the first 100 ms after stimulus onset (measured in arbitrary units of mV). pp = prepulse intensity in db (SPL). The startle stimulus was 115 dB.

Forced swimming test

Treatment with fluoxetine or paroxetine did not affect time spent immobile in the forced swimming test (Figure 10).

Corticosterone

Treatment with fluoxetine or paroxetine did not affect corticosterone levels (Figure 11).



Conclusion

Administration of fluoxetine to rats during adolescence did not have an effect on their performance on 3 tests of emotional behaviour and sensory gating. However, consistent with the de Jong study, paroxetine administration to rats during adolescence resulted in anxiogenic behaviour as demonstrated in the elevated plus-maze test.

Assessor's comments

Fluoxetine administered to rats of an equivalent age to that of the indicated patient population had no effect on the behaviour of rats in the measures of anxiety conducted in the study. The statistically significant dose-dependent reductions in acoustic startle response in rats previously reported by [REDACTED] 2004 and the behavioural changes in mice reported by Ansorge et al 2004 was not reproduced in the present study.

In contrast treatment with the comparator paroxetine at the top dose of 17 mg/kg/day induced a statistically significant reduction in the number of head dips in the elevated plus maze test, a test of anxiogenic behaviour, and reduction in the length of time spent in the open arm of the maze (not statistically significant) performed during the dosing period thus confirming (a) the responsiveness of the test system and (b) the reproducibility of the anxiogenic effect of paroxetine reported in the study by de Jong et al 2006

In the open literature studies conducted with fluoxetine have either showed it to be anxiogenic, anxiolytic or have no effect on emotional behaviour depending on study design. The reasons for these contrasting responses are unclear but it may be due to the sensitivity of the test system, species and strain differences and dose.

Currently the preclinical section of the SPC cites the findings of the De Jong study and it is recommended that the MAH submit a type II variation to update the SPC statement with the latest findings on the behavioural effects of fluoxetine as follows:

Current

5.3 Preclinical safety data

Another study in juvenile mice (treated on postnatal days 4 to 21) has demonstrated that inhibition of the serotonin transporter had long-lasting effects on the behaviour of the mice. There is no information on whether the effect was reversible. The clinical relevance of this finding has not been established.

Proposed

5.3 Preclinical safety data

*Another study in juvenile mice (treated on postnatal days 4 to 21) has demonstrated that inhibition of the serotonin transporter had long-lasting effects on the behaviour of the mice. There is no information on whether the effect was reversible. The clinical relevance of this finding has not been established. **No behavioural effects were observed in a juvenile study in rats following treatment with fluoxetine.***

IV. CONCLUSION

Fluoxetine is the only SSRI indicated for use in the paediatric population therefore the present study was designed to investigate the effects of fluoxetine in rats of an equivalent age of the indicated paediatric population. The absence of effects on behaviour in the animals is reassuring as it showed a lack of reproducibility of the findings reported in the study by █████ 2004 and also showed no other adverse effects relevant to this patient population.

The comparator paroxetine did induce effects at the doses tested but also did not cause an increase in impaired acoustic startle response. The open literature shows both anxiolytic and anxiogenic responses of the SSRIs in juvenile animals and this conflicting data makes it difficult to determine the relevance of the findings to the patient population. Therefore, until more information is generated on mechanisms the relevance to man is unknown

The MAH will be requested to submit a type II variation to update the SPC with the findings of the present study as discussed.

V. REFERENCES

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2. █████ (2003) Oral (gavage) fertility and general reproductive toxicity study (FGRP) of fluvoxamine maleate in rats. Argus Research Report No. TX.114.07.06P CRO
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6. Stuhler, J. (2002) Toxicokinetic Assessment for the oral (gavage) dosage-range fertility and developmental toxicity study of fluvoxamine maleate in female rats. Quintiles. Prepared for Solvay Pharmaceuticals. Report number TX.114.07.07CRO
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VI. ANNEXE

ANNEX 1: EMOTIONAL BEHAVIOUR (CHMP REPORT – for information)

The MAH considered the available non-clinical data acceptable for the assessment of potential effects on brain development and function. The juvenile rat study included a comprehensive evaluation of brain development and function designed to investigate potential effects on CNS development during human childhood through adolescence.

Evaluations included sensory and motor functions, learning and memory in a complex water maze, and brain histopathology. Although subtle decreases in startle amplitude were noted 2 weeks after completion of fluoxetine treatment, there were no differences when rats were retested 4 weeks later. There were also no changes in latency to respond to auditory startle at either evaluation. Non-reversible changes in startle and learning (females only) only occurred in conjunction with severe systemic toxicity. Thus, the persistent effects on startle are confounded by the clinical condition of the animals (██████ 2004).

Although a recent study reported long lasting behavioural changes after fluoxetine treatment in 5-HTT+/+ and 5-HTT+/- mice (Ansorge et al 2004), the clinical relevance of these findings for childhood exposure is questionable. In that study, 5-HTT+/+, 5-HTT+/-, and 5-HTT-/- mice were administered saline or fluoxetine from postnatal day (PND) 4 through 21. This period of brain development is considered equivalent to a human third trimester foetus through a 2-year old child (Anderson 2003; Bayer et al. 1993; Kimmel and Buelke-Sam 2001; Rice and Barone 2000; Rodier 1980) and does not replicate the recommended age range for fluoxetine administration.

The MAH argued that the application of any animal model to evaluate emotional behaviour and the relevance of those non-clinical data to a depressed child is exceedingly problematic. A non-clinical model cannot account for the underlying disease state of depression that is reflected by alterations in emotional behaviour in this depressed patient population. Any behavioural changes observed in a normal animal could not be extrapolated to humans with clinical conditions requiring treatment to positively change “emotional” behaviours.

Animal models of depression and anxiety are most widely used as screening tools in the search to identify new compounds with therapeutic potential and are not included in safety assessment batteries. The effects of known anxiolytic drugs, including serotonergic drugs, have been inconsistent across these different behavioural paradigms (Silva and Brandao 2000), resulting in the need for better, more predictive models to assess emotional behaviour (Cryan et al. 2002).

Furthermore, important interindividual variations in rodent responses on tests of anxiety and depression have been demonstrated (Hinojosa et al. 2006) and suggest that outcome data are highly dependent on study design (for review of factors involving variability in 5-HT animal studies see Griebel 1995). For example, depending on the study design, fluoxetine has been reported to have no effect on emotional behaviour, to be anxiogenic, and to be anxiolytic in Wistar rats (Griebel 1995). Additionally, the majority of behavioural models used to assess antidepressant and anti-anxiety activity in rodents are sensitive to strain differences (Cryan et al. 2002; Tang and Sanford 2005), suggesting that strain comparisons might be needed to avoid false negatives and/or false positives.

In a juvenile toxicity study with fluoxetine, a comprehensive behavioural battery meeting ICH S5A guidelines was conducted. Behavioural batteries such as this, where sensory, motor, and cognitive development are evaluated, have demonstrated intralaboratory and interlaboratory reliability as well as detection sensitivity (██████ 1985). The demonstrated reliability of these behavioural assessments makes these batteries valuable and appropriate in safety assessment. The behavioural evaluations conducted in the MAH’s juvenile toxicity study included locomotor activity in an open field. Assessment of open field activity includes the assessment of behavioural components of arousal,

novelty seeking, fear responses, and stereotype which may be associated with fear or anxiety in response to the novel environment and/or to the natural aversion of rodents to open spaces. There were no effects of fluoxetine on the open field in the juvenile toxicity study with fluoxetine.

Although two published papers (Ansorge et al. 2004; de Jong et al. 2006) suggested anxiogenic effects of some SSRIs in rodents, these effects were minor, specific to individual assessments, and/or not relevant to the patient population. The Ansorge study used a genetically-manipulated, transgenic mouse model with a dosing period considered equivalent to a human third trimester fetus through a 2-year-old child, thus eliminating any predictability for effects of fluoxetine in children and adolescents. The de Jong study was well-designed and incorporated multiple evaluations of anxiogenic behaviours in adult rats administered with fluvoxamine and paroxetine. Although a comprehensive series of behavioural tests was conducted in this study, only mild effects occurred in one of the tests. Thus, these effects could also be due to a temporary effect of the discontinuation of the drug treatment rather than to a permanent change in the brain.

In order for a behavioural study to be useful for safety assessment, the methods must have a strong predictive validity with reliable and robust responses within and between laboratories (Cryan et al. 2002).

The CHMP agreed with the MAH that the effects on the startle response were small and reversible at a reasonable dose. The rats were tested also in a maze and no statistically significant results were reported. Effects were mainly (although not exclusively) in the high dose group. Dosages leading to an extensive weight loss cannot be trusted to lead to relevant conclusions.

Furthermore, the CHMP agreed that the study in genetically different strains of mice is not focused on the right time window (the period of brain development in the mice was considered equivalent to a human third trimester foetus through a 2-year old child, thus markedly different from the intended population) and the clinical relevance of the data in knock-out mice in Ansorge et al, for the intended population is likely to be small.

Despite the difficulties that exist in evaluating the clinical relevance of any potential long-term changes in the developing brain, the CHMP considered that emotional development warranted further exploration, taking account the paucity of available data and the difficulties that exist in studying potential long-term neurobehavioural effects of fluoxetine in humans. The CHMP recommended that a further pre-clinical study should be performed and the design of the study should be such that the age/development phase of the animals when exposed is as closely matched to the development of the brain in the target paediatric population. The focus should be on assessing measures of anxiety.

The MAH committed therefore to study the characterization of effects on specified emotional behaviours in juvenile rats. In this study, fluoxetine would be administered to CD rats from postnatal day 33 to postnatal day 62 with evaluations in the elevated zero maze, forced swimming test and prepulse inhibition test, once during treatment and 2 months post-treatment.

